6. OMOE. Organic vs. inorganic arsenic in selected food samples. Report No. 87-48-45000-057. Toronto, Ontario:Ontario Ministry of the Environment, Hazardous Contaminants Coordination Branch, 1987.
7. Sanders JG. The concentration and speciation of arsenic in marine macro-algae. Estuarine Coast Mar Sci 9:95–99 (1979).
8. Pyles RA, Woolson EA. Quantitation and characterization of arsenic compounds in vegetables grown in arsenic acid treated soil. J Agric Food Chem 30:866–870 (1982).
9. OMOE. Draft criteria document for arsenic standards. Toronto, Ontario:Ontario Ministry of the Environment, Hazardous Contaminants Coordination Branch, February 1987.
10. Guthrie HA. Introductory nutrition. St. Louis, MO:C.U. Mosby Company, 1983.
11. Epstein Y, Sohar E. Fluid balance in hot climates: sweating, water intake, and prevention of dehydration. Public Health Rev 13:115–137 (1985).
12. Hubbard RH, Armstrong LE. The heat illnesses: biochemical, ultrastructural, and fluid electrolyte considerations. In: Human performance physiology and environmental medicine at terrestrial extremes (Pandolph KB, Sawka MN, Gonzalez RR, eds). Indianapolis, IN:Benchmark Press, 1988;305–359.
13. Greenleaf JE, Averkin EG, Sargent F II. Water consumption by man in a warm environment: a statistical analysis. J Appl Physiol 21:93–98 (1966).
14. Szlyk PC, Hubbard RW, Matthews WT. Mechanisms of voluntary dehydration among troops in the field. Mil Med 152:405–407 (1987).
15. Szlyk PC, Sils IV, Francesconi RP, Hubbard RW. Patterns of human drinking: effects of exercise, water temperature, and food consumption. Aviat Space Environ Med 61:43–48 (1990).
16. Pitts GC, Johnson RE, Consalazio FC. Work in the heat as affected by intake of water, salt, and glucose. Am J Physiol 142:253–259 (1944).
17. U.S. Army Research Institute of Environmental Medicine. Sustaining health and performance in the desert: a pocket guide to environmental medicine for operations in Southwest Asia. Technical Note No. 91-2. Natick, MA:U.S. Army, 1990.
18. Galagan DJ, Vermillion JR, Nevitt GA, Stadt ZM, Dant RE. Climate and fluid intake. Public Health Rep 72:484–490 (1957).
19. Yarbrough BE, Hubbard RW. Heat-related illnesses. In: Management of wilderness and environmental emergencies (Auerbach Geehr, ed). Mosby Year Book, 1988;119–143.
20. Sawka MN, Pandolph KB. Exercise-heat strain during hypohydration: interaction with heat acclimation and aerobic fitness. Presented at the Fourteenth Commonwealth Defense Conference on Operational Clothing and Combat Equipment, Australia, 1985. NTIS AD-A151 023. Springfield, VA:National Technical Information Service, 1985.
21. Rothstein A, Adolph EF, Wills JH. Voluntary dehydration. In: Physiology of man in the desert (Adolph EF, ed). New York:Interscience Publishers, 1947;254–270.
22. Abernathy CO, Marcus W, Chen C. Report on arsenic (As) work group meetings [Internal memorandum to P. Cook and P. Preuss]. Washington:Environmental Protection Agency, Office of Drinking Water, 23 February 1989.
23. Brown KG, Abernathy CO. The Taiwan skin cancer risk analysis of inorganic arsenic ingestion: effects of water consumption rates and food arsenic levels. In: Proceedings of the Society for Environmental Geochemistry and Health Second International Conference on Arsenic Exposure and Health Effects, San Diego, California, 12–14 June 1995 (Chapell WR, Abernathy CO, Calderon R, eds). London: Chapman & Hall. (In press).
24. Hopenhayn-Rich C, Smith AH, Goeden HM. Human studies do not support the methylation threshold hypothesis for the toxicity of inorganic arsenic. Environ Res 60:161–177 (1993).
25. Froines J. Studies of arsenic ingestion from drinking water in northeastern Taiwan: chemical speciation and urinary metabolites. Presented at the Workshop on Arsenic Epidemiology and PBPK Modeling, Annapolis, Maryland, 27–28 June 1994.
26. Del Razo LM, Hernández JL, García-Vargas GG, Ostrosky-Wegman P, de Nava CC, Cebrián ME. Urinary excretion of arsenic species in a human population chronically exposed to arsenic via drinking water, a pilot study. In: Arsenic exposure and health (Chappel WR, Abernathy CO, Cothern CR, eds). Northwood, England:Science and Technology Letters, 1994;91–100.
27. Farmer JG, Johnson LR. Assessment of occupational exposure to inorganic arsenic based on urinary concentrations and speciation of arsenic. Br J Indust Med 47:342–348 (1990).
28. Hopenhayn-Rich C, Biggs ML, Smith AH, Moore LE, Kalman DA. Arsenic exposure in a chronically exposed population: a multifactorial analysis of metabolite distribution. Presented at the Society for Environmental Geochemistry and Health Second International Conference on Arsenic Exposure and Health Effects, San Diego, California, 12–14 June 1995.
29. Hseuh YM, Huang YL, Wu WL, Huang CC, Yang MH, Chen GS, Chen CJ. Serum β-carotene level, arsenic methylation capability and risk of skin cancer. Presented at the Society for Environmental Geochemistry and Health Second International Conference on Arsenic Exposure and Health Effects, San Diego, California, 12–14 June 1995.
30. Yamauchi H, Takahashi K, Mashiko M, Yamamura Y. Biological monitoring of arsenic exposure of gallium arsenide- and inorganic arsenic-exposed workers by determination of inorganic arsenic and its metabolites in urine and hair. Am Ind Hyg Assoc J 50:606–612 (1989).
31. Del Razo LM, García-Vargas G, Vargas H, Albores A, Cebrián ME, Montero R, Ostrosky P, Kelch M. Chronic high arsenic exposure alters the profile of urinary arsenic metabolites in humans. The Toxicologist 15:262 (1995).
32. Hughes MF, Menache M, Thompson DJ. Dose-dependent disposition of sodium arsenate in mice following acute oral exposure. Fundam Appl Toxicol 22:80–89 (1994).
33. Valentine JL, Kang HK, Spivey G. Arsenic levels in human blood, urine, and hair in response to exposure via drinking water. Environ Res 20: 24–32 (1979).
34. Eaton AD. Determining the practical quantitation level for arsenic. J Am Water Works Assoc 86:100–114 (1994).
35. Irgolic KJ. Arsenic. In: Techniques and instrumentation in analytical chemistry, hazardous metals in the environment, vol 12 (Stoeppler M, ed). Amsterdam:Elsevier Science Publishers, 1992;287–350.
36. Vahter M, Concha G, Nermell B, Nilsson R, Dulout F, Natarajan AT. A unique metabolism of inorganic arsenic in native Andean women. Eur J Pharmacol 293:455–462 (1995).
37. Vahter M, Marafante E. Effects of low dietary intake of methionine, choline, or proteins on the biotransformation of arsenite in the rabbit. Toxicol Lett 37:41–46 (1987).

Response: Accuracy, Arsenic, and Cancer

Slayton et al. (*1*) have attempted to rebut portions of our *EHP* commentary on arsenic (As) cancer risk assessment (*2*) and some of the debate that has emerged over such assessments. In responding to Slayton et al., we offer some additional information that interested readers may find helpful in comparing our commentary and these responses with Slayton et al. Our commentary drew particular attention to some obvious problems in reported criticisms of the Taiwanese As exposure and carcinogenesis data.

Dietary versus drinking water As in the Taiwanese study population. The cancer slope factors for ingested As based on well water As levels in Taiwanese studies were challenged by Beck et al. (*3*) and Yost et al. (*4*). They argued that their finding of inorganic As in some rice and yam samples from Taiwan required that diet As be factored into derivation of cancer dose–response curves. We noted some quantitative questions about their results. Our concerns are not trivial and the Yost et al. findings (*4*) require toxicological context and some mention of the wider implications. Nowhere in our paper did we argue that no inorganic As could be present in their few samples, merely that the As fractions in Yost et al. (*4*) appeared to differ in some cases from other reported values.

We suggested, as one possibility, use of different analytical methods. We also indicated that any analytical methodology involving use of strong acids merits scrutiny when speciating multiple forms of an element like As, especially when significant fractions of a carcinogenic form are being reported. How well do such measured levels of As forms reflect the original sample forms, and how well does *in vitro* chemical behavior reflect *in vivo* disposition of these forms when ingested? Inorganic As might be liberated in analysis but not *in vivo* when ingested. Water As does not offer this problem, being typically in the inorganic form. Such differences for rice, yams, and similar foods can be studied with controlled-diet feeding studies in the same way that seafood and other marine biota were studied.

There are several wider potential implications of the Yost et al. data (*4*), as reported. They may indicate the need for speciation analyses to detect variable inorganic As content when doing risk assessments at other As-impacted areas and communities. There may be a need to pay much more attention to food crops as exposure pathways for As in these communities. Use of analytical methods that do not methodologically alter original biochemical forms might be necessary.

Slayton et al. (*1*) note the well-known fact that strong acids do not materially break

down organoarsenicals in seafood and they assume that any organoarsenicals in food items of the type they analyzed would behave likewise, i.e., inorganic As is not artifactually generated. The forms and chemical behavior of organoarsenicals in seafood may not be relevant to forms of As in their sample types . Even within marine biota, there are major differences in isolable organoarsenicals. Seafood yields mainly acid-stable arsenobetaine and lesser levels of acid-stable arsenocholine (*5,6*). Marine algae yield water-soluble organoarsenicals such as 5–dimethylarsinoyl derivatives of 5-deoxyribosides (*5-7*) and lipid-soluble forms that appear to include phospholipid derivatives of these arsenosugars (*5,8*). Le et al. (*9*) recently showed partial metabolic breakdown of an arsenosugar to dimethylarsenic (DMA) in a kelp product, nori, compared to less breakdown of seafood As. Isolable arsenicals are highly variable across biota and require caution in assumptions about comparative behavior.

Slayton et al. accuse us of greatly mischaracterizing data in two cited sources, a paper by Pyles and Woolson (*10*) and a Canadian report on As speciation in food items summarized in an EPA report (*11,12*). We correctly calculated from Table 1 of Pyles and Woolson (*10*) that the inorganic As content for potato flesh in the third column (0.1 ppm) was 8% of the total As content of potato flesh reported in column 7 (1.19 ppm). The basis of the incorrect value in Slayton et al. for potato flesh is a puzzle. The closest calculation to this value for potato flesh relates the inorganic As value of 0.1 ppm in column 3 to the As total for just one of three fractions of the total As, i.e., the total As of 0.37 ppm in column 2 for the methanol-water fraction, giving about 28%. There are also the chloroform (column 5) and the nonextractable (column 6) phases, both presumably representing organoarsenicals of varying structure and solubility. These latter two fractions contain 69% of the total As for potato flesh in column 7. People ingest all of the As that may be present in their diet, not just that As fraction present in analytical methanol–water extracts.

We also did not mischaracterize the percent of inorganic As in rice or potatoes in the cited Canadian report (*11*) as they were summarized in a 1988 EPA report on arsenic (*12*). Table E-1, Appendix E of this EPA report (*12*) clearly states that rice and potatoes contained 35% and 10% inorganic As, respectively. We state the same values in our paper. Data in the Canadian report were provided to EPA by Roland Weiler, a now-retired member of the contaminant standards staff in the Ontario, Canada, environment ministry and a member of the external advisory group that helped assemble the 1988 EPA report. Slayton et al. refer to a Canadian source as not being familiar with inorganic As in potatoes and claim a mix-up in documents. We have no explanation for any claimed differences in information on potato analyses nor can we say information in the EPA report is incorrect. Neither can Slayton et al. note that the Pyles and Woolsen report (*10*) did not include data on rice. This is correct. We more precisely intended to compare reported data for potatoes and not to note any rice data in Pyles and Woolson.

We would add an important statistical and epidemiological point ignored by Beck et al. (*3*), Yost et al. (*4*), and Slayton et al. in discussing their rice and yam data. The Beck et al. (*3*) and Yost et al. (*4*) arguments are based, as we understand it, on a first sampling of a half dozen rice and yam samples combined. There was also a recent second sampling (*13*) consisting, we understand, of 20 samples. Such small samplings are relatively meaningless for quantitatively characterizing historical dietary As exposures of the huge Taiwanese exposure population studied by Tseng and coworkers (*14,15*). That risk population was 103,514 persons, of whom 40,421 subjects in 37 villages were studied in a door-to-door survey (*15*). A total of 142 water samples from 114 wells throughout these villages were tested.

Slayton et al. are incorrect in saying we erred in noting that Beck et al. (*3*) excluded As in cooking water for rice as a diet As source because EPA already takes water used for cooking food into account when estimating water intakes. EPA's accounting for food-cooking water in its estimates of total Taiwanese water intake is well known and the 1-liter estimate has been used since at least the 1988 EPA report (*12*). Assigning As in food preparation water, as EPA does, to total water consumption rather than to As in diet seems logical when the sole focus is total impact of water As. Water As incorporated into the diet by cooking or other means and ingested with a meal may not have the same bioavailability (absorption rate) as As in water or beverages consumed on an empty stomach. This potentially alters the overall daily water As absorbed dose. Furthermore, ingestion rates of water As incorporated in cooked foods will vary with food consumption rates rather than with water intake rates. Water As, when drunk directly or in beverages, is appropriately assumed to be totally absorbed (*12,16*), and such water As intake as it is used in unit lifetime cancer risk or cancer slope factor derivations is equivalent to absorbed dose. Better defined analyses of both water and diet arsenic intakes in the present context would arguably result from separating out food preparation water As from drinking and beverage water As.

The response of Slayton et al. (*1*) to our comment about how food As would affect one case, an illustrative linear (regression) dose–cancer response curve based on water As intakes as noted by Smith et al. (*17*), seemed irrelevant. They labeled this example of ours as incorrect because their choice, among various curve forms still being debated, differed from ours. This is hardly an adequate basis. In our example, a positive slope intercept represents the rate from nonwater As sources. Any added, relatively stable food As intake in such a linear relationship shifts the Y intercept further up the ordinate. The slope remains intact.

Water ingestion rates in As-exposed Taiwanese. We drew attention to the EPA's quite arbitrary use of a total daily water intake of 4.5 liters among the exposed Taiwanese, a value supported by Beck et al. (*3*). We pointed to the need for actual water consumption studies in Taiwan and underscored this need by citing studies showing that various populations under diverse heat stress conditions drink less water than they should. While any reliable estimate of daily Taiwanese well-water consumption still eludes accurate determination, we also appreciate an alternative view that any arguments over water volume intakes may be much ado about little. That is, a twofold or so difference in water intake may well be less significant than other potential sources of variability and uncertainty in any uncertainty analysis of As cancer risk assessment models.

Little of the Slayton et al. response refutes our principal point, and they offered inaccurate or confused criticisms of our discussion of this topic. Our use of the Guthrie estimate of adult water intakes (*18*) was to show the good agreement between estimated and actual intakes measured in the late 1970s for a large number of American adult women with diverse physical activities who were part of the U.S. Department of Agriculture Nationwide Food Consumption Survey (*19*). Slayton et al. cite a very diverse mix of studies, some of them cited by us as well, indicating that people should drink more water than they do and should follow water intake guidelines, that they may go in and out of voluntary dehydration, and that people in hot areas sweat. This is not new. The fact remains that actual field studies of chronically heat-stressed individuals show people consume less water than they should. For example, the ignored studies of Kristal-Boneh et al. (*20*) for adults and Phillip et al. (*21*) for young children show less water consumption than expected, casting doubt on arbitrary selections of water intake volumes.

Slayton et al. criticize our use of the Galagan et al. study (*22*) in which California children were studied in terms of fluid intake

as a function of ambient temperature. We do not understand their problem. The Galagan et al. paper (*22*) clearly states that the daily maximum temperature was used because the maximum was as accurate as five other forms of reportage in depicting water intake rates and is also the measurement most commonly recorded in weather data. Slayton et al. claim that drinking water was only part of fluid intakes in these children. Of course; and the same can generally be said for Taiwan as we discussed in detail in our paper. Finally, Taiwanese and California children both encounter an ambient temperature cycle, with some maximum temperature and cooler periods.

As measurement and intake-dependent toxicokinetics. We took strong issue with the argument that data in the 1979 study of Valentine et al. (*23*) show As intake-dependent toxicokinetics and cited both analytical methodological and metabolic reasons. A critical look at the methodological problems was greatly assisted by former directorship of laboratory development and application of trace and ultra-trace methods for such toxicants as arsenic in blood and other media during the time period of the Valentine et al. paper (*24–26*). We noted that method limitations make meaningless their reported data on blood As for estimating As toxicokinetic alterations.

Slayton et al. (*1*) apparently still support the Valentine et al. study (*23*), but their arguments are incorrect. The Valentine et al. method, as described in the 1977 Kang and Valentine paper (*27*), employed quite early atomic absorption spectrometry (AAS) instrumentation, a Perkin-Elmer Model 303 atomic absorption spectrometer in the flame mode, As hydride generation from mineralized samples, either continuous flow or gas balloon sampling transfer modes, and 5 ml blood samples. Their reported detection limit was 0.02 µg (20ng)/total analytical sample for all media analyzed (*27*). Each of their blood As analytical samples represented 5 ml of blood. This corresponds to 4 ppb blood As (4 µg/l). The 1977 comprehensive and contemporaneous review by Braman (*28*) for quantitative analysis of various As species via hydride generation summarized various papers showing sensitivity for flame AAS analysis of As after hydride generation is a lower detection limit of 5 ppb on a concentration basis, similar to the reported Kang and Valentine value (*27*).

A detection limit is not a quantitation level, which is the level at which one can reliably or accurately quantitate the As in a sample. The quantitation level is some multiple of the detection limit. A reliable quantitation limit (RQL) would be no less than a fivefold multiple of this 4-ppb detection limit for very good current laboratory performance (*29*). The 1994 methods review of Irgolic (*30*) provides a detailed discussion of detection versus quantitation limits and argues for a quantitation limit as being no less than 10-fold higher than the detection limit. Experience with the topic of quantitative methodology would certainly support Irgolic's statement, particularly as it applied to 1970s methodology and the Valentine et al. (*23*) proficiency level therein. Overall, the reliable or accurate quantitation limit for Valentine et al. (*23*) methodology would be either within a range of 20–40 ppb or 40 ppb, the latter based solely on Irgolic (*30*). These limits are up to 13-fold higher than the 3–5-ppb quantities that Slayton et al. (*1*) suggest were being reliably or accurately measured as As exposure group means in the Valentine et al. study (*23*). Put differently, Slayton et al. would have to assume that Valentine et al. were reliably or accurately quantitating As content of blood from most of their exposure groups at or below their reported detection limit (4 ppb) and up to 13-fold below their quantitation limit.

Slayton et al. also offer a number of comments about detection limits for various methods in the 1970s and methods of the type used by Valentine et al. (*23*), citing various sources. These comments are moot, given the reported Valentine et al. detection limit discussed above. We are confident that their cited review by Irgolic (*31*) would not conflict with the stated detection limit or the Braman review (*28*). One reference to extremely low As detection limits in blood and other biological media in Slayton et al. [Eaton et al. (*29*)] appears wrongly cited.

The idealized case of Slayton et al. and its claimed ability to outperform collective results from proficiency surveys are not fully relevant to the paper at issue. We also note that many laboratories performed much more poorly than desirable. Poor performance at the trace and ultra-trace level was quite common in the 1970s and earlier. Proficiency testing programs were created by professional and public organizations to identify and improve such lagging laboratory performance. We viewed the Eaton proficiency survey (*29*) not only as providing a comparison for Valentine et al. (*23*) but also as serving to ascertain whether current proficiency, as defined by today's criteria, permits quantitative measurements of As in blood or other media at very low exposure levels.

We stand by our earlier conclusion that the 3–5 ppb levels for As, cited as group means for four of their five groups in the Valentine et al. study (*23*), were at or below their detection limits and were associated with little more than background noise. Any conclusions drawn from that study based on blood As and arguing for altered toxicokinetics at low water As intakes are invalid. We also believe that urine As–water As relationships in Valentine et al. are more toxicokinetically useful.

The role of MMA:DMA ratios in quantifying carcinogenic risk. Our paper took issue with the view that any increase in the ratio of urinary MMA to DMA with increase in As exposure heralds increased cancer risk. Reasons were stated and are not repeated here. Little has been clarified on this topic since our commentary appeared, including the point that changes in the one known carcinogenic form, inorganic As, are not clearly linked to the above ratio changes in studies reporting such changes.

Slayton et al. do not provide convincing evidence that alterations in the ternary inorganic As–MMA–DMA relationship are quantitatively linked to cancer risk characterization or that the biomolecular mechanisms of As carcinogenesis compel acceptance of increased cancer risk when the MMA and DMA forms begin to undergo moderate change in relative distribution. They cite some new conference presentation material and an older occupational exposure study by Yamauchi et al. (*32*). With regard to the chronic high inorganic As exposures in occupational settings and methylated As urinary profiles in workers, the 1986 study of Vahter (*33*) showed that the percent inorganic As in smelter workers' urine samples was virtually identical to the inorganic fraction of urines of a nonworker population of nonfish eaters, 18 and 19% respectively. We calculated the corresponding ratios of percent MMA to percent DMA as 0.25 and 0.33, respectively, a quite modest change. Slayton et al. note that cutaneous changes were linked to changes in MMA:DMA ratios in the conference presentation of Del Razo et al. (*34*), which we also discussed. This finding requires further clarification and peer-review publication as to the nature of the skin changes and strength of the association with metabolites and, especially, relationship to the urinary inorganic As content.

Slayton et al. cite a 1995 As conference presentation by Hopenhayn-Rich et al. (*35*) as showing an increased MMA:DMA ratio in exposed northern Chilean subjects; however, the full paper by these investigators (*36*) makes clear that interindividual ratio differences were within a much greater range than were differences between exposure groups. Of importance to our view, the exposed versus control fractions of inorganic As were not significantly different (18.4 versus 14.9%) and not materially different, as the authors note, from the variability seen in lower exposure data from various other studies. The exposed group had water levels of about 600 µg/l. The

relative difference in the MMA:DMA ratio for the exposure versus control groups as described by these authors in their Table 2 is 1.6. This ratio of ratios is identical to what we calculated for the corresponding relative difference for a study of As exposed subjects in Nevada (*37*) discussed below, using percentages of forms as calculated in our commentary. This lack of change exists despite the fact the mean water As for the Nevada exposures was almost twice the Chilean level.

Slayton et al. ignored a relevant 1994 paper cited in our commentary, Warner et al. (*37*). We discussed the distributions of inorganic and methylated As forms in control and exposed subjects. Exposure was to quite high water As concentrations (mean = 1312 µg/l), while the corresponding MMA:DMA ratios for exposed and control subjects were 0.32 and 0.50, which, given the small sample size and variance, are not striking differences. These values are for a study in which the mean exposure (1132 µg/l) was about 50% higher than the EPA's estimated Taiwanese mean water exposure level for the high exposure group [800 µg/l (*12*] and, as noted by Warner et al. (*37*), well above those elevations reported for other American water As-impacted communities. Slayton et al. cite an animal acute dosing study by Hughes et al. (*38*), which they note shows dramatic evidence of dependence of MMA:DMA ratios on dose. Beck et al. (*3*) also cited this paper; we have already rebutted Beck et al.'s argument by noting that it had little relevance to chronic human exposures.

One difficulty with assigning a cancer risk prediction role to elevated urinary MMA:DMA ratios is the fact that this ratio can increase through any one of at least four possible metabolic scenarios. Only one of these seems to offer a readily interpretable linkage to increased cancer risk with increasing As exposure if one uses distributions of urinary As forms. This assumes that levels of all urinary As forms, especially those of inorganic As, are toxicokinetic correlates of what is going on *in vivo* in terms of carcinogenic mechanisms. If one argues that this does not apply to inorganic As, then the MMA:DMA ratio becomes problematic as well. The case in point is one in which the fractional amount of inorganic As shows a net increase with elevated exposure, along with necessary net reductions in the fractions of both MMA and DMA. A higher inorganic As fraction in the body increases cancer risk. However, the extent of net decline of MMA would be proportionately less than that of DMA, owing to a build-up of MMA substrate in a sequential methylation pathway where DMA formation from MMA occurs and where DMA formation is relatively more inhibited by As than MMA formation. Such a differential sensitivity to arsenic inhibition has been proposed (*39*). One should then see a higher fraction of inorganic As with increased As intake; a higher fractional MMA:DMA ratio; a lower fractional MMA:MMA ratio in exposed versus less exposed; a lower fractional DMA:DMA ratio in exposed versus controls; and a significantly reduced sum of fractional MMA + DMA for exposed versus nonexposed.

Current studies of elevated environmental As that report urinary metabolites can be typified by the north Chile report (*36*). Here, in exposed subjects, one sees little increase in fractional inorganic As; a moderately elevated MMA:DMA ratio; a lower DMA:DMA ratio; an elevated MMA:MMA ratio compared to controls; and little change in MMA + DMA compared to controls. These observations mainly suggest the second of the possible metabolic scenarios, that is, the As methylation capacity in these exposed Chileans can still deal with increased inorganic As entering the body (little increased inorganic fraction), but more of the As methylation process is handed off to MMA formation and less to DMA biosynthesis (increased MMA:DMA and MMA:MMA; decreased DMA:DMA; little change in MMA + DMA). A quite similar pattern is seen with the Nevada exposure study, using our commentary calculations. At this time, a change in the MMA:DMA ratio still appears to be a metabolic distinction without a documented cancer risk difference.

Taiwanese diet methyl donors and implications for Taiwanese cancer rates. Our commentary noted that Taiwanese nutritional deficiencies in terms of methyl sources cannot be identified and cited data showing nutritional sufficiency. Using the data for diet methyl donors in the Taiwanese, we calculated a small allocation of diet methyl intake to As biomethylation, an average of ~0.7%, or a ratio of intake to As methylation requirement of about 150. This indicates a trivial requirement for biomethylation of As at high intakes when adequate intake of methyl sources exists. The ratio is obviously not a quantitative reflection of the status of any specific methyl pools in the body, e.g., those in the liver and other organs involved in As biomethylation. We can reasonably assume, however, that metabolic pools involved in As biotransformation are furnished adequate methyl levels when diets are replete in methyl sources.

Slayton et al. challenged the value of our calculated percentage and ratio by arguing that an animal feeding study using restricted methyl donor diets (*40*), a study discussed by us as well, shows that the percentage of methyl groups allocated to As methylation is not a useful indicator for anything. Their arguments are not convincing. We only addressed the specific case of the nutritional status of the Taiwanese in which the ratio of dietary methyl loading to the average As methylation requirement was about 150. They claim that ratios of 67–100 in the rabbits were linked to reduced methylating capacities; that may well be the case. We never said or implied that such ratios would not be linked to a reduction of methylation capacity. We also note that their calculated ratios of 67 and 100 in the rabbits are only 45% and 67% of the 150 we estimated for an average of the high As exposure group for methyl donor-adequate Taiwanese. These could be significant decrements in terms of multiorgan methyl pool changes.

Their comments, however, indirectly suggest to us a means to more precisely relate dietary methyl status to metabolism of As. One can expand the Vahter and Marafante approach (*40*) to include studies with a much larger number of diet methyl loadings and As exposures. Any resulting changes in urinary metabolite profiles from use of a wide range of ratios of methyl availability to As methylation might be useful for modeling onset of altered methylation, the rate of altered methylation, the toxicokinetic nature of the associated curves, etc. Any such change can be viewed as factorial: dietary methyl being variable with stable As exposure, fixed diet with As exposure being variable, or both changing together. Slayton et al. state that methylation of As in the rabbit study of Vahter and Marafante (*40*) requires 0.3% of the methyl donor intake of donor-replete diets to methylate the As dose, equivalent to a ratio of ~330. The corresponding Slayton et al. estimate of a ratio of 77 in restricted methyl intake animals is associated with a 40% decline in DMA (65% to 39%). Therefore, a fourfold decline in the methyl intake–As methylation ratio from 330 to 77 produced a 40% DMA decline. Onset of altered DMA formation occurs within this range for this particular set of test conditions.

Is such an approach relevant to the environmental epidemiology of nonoccupational populations with chronic As exposure? This is not clear. MMA and DMA distributions in animals differ to some degree from that in humans, the latter having more MMA (*41*). There are also the questions of whether altered ratios of MMA to DMA universally occur with exposure changes and what the relevance is of such ratio changes to cancer risk, as discussed above. As a method for clarifying some issues, however, we believe it would be of interest to carry out careful studies of the individual and group nutritional status in those populations currently being studied, in terms of daily dietary methyl intakes and the relationship of such intakes to

both water As exposure levels (ratios) and urinary As profiles. Our ratio of approximately 150 for the highest exposure mean (800 µg/l) in the earlier Taiwanese subjects could be a point of departure in such studies.

Paul Mushak
PB Associates
Durham, North Carolina

Annemarie F. Crocetti
Consultant in Nutrition Epidemiology
New York, New York

Reference

1. Slayton TM, Beck BD, Yost LJ, Schoof RA, Gauthier TD, Reynolds KA, Chapnick SD, Jones L, Valberg PA. Issues in arsenic cancer risk assessment [letter]. Environ Health Perspect 104: 1012–1014 (1996).
2. Mushak P, Crocetti AF. Risk and revisionism in arsenic cancer risk assessment. Environ Health Perspect 103:684–689 (1995).
3. Beck BD, Boardman PD, Hook GC, Rudel RA, Slayton TM, Carlson-Lynch H. Response to Smith et al. [letter]. Environ Health Perspect 103:15–17 (1995).
4. Yost L, Schoof RA, Guo HR, Valberg PA, Beck BD, Crecelius E, Greene E, Bergstrom P. Recalculation of the oral toxicity values for arsenic correcting for dietary arsenic intake. Presented at the Society for Environmental Geochemistry and Health Rocky Mountain Conference, Salt Lake City, Utah, 18–19 July 1994.
5. Morita M, Edmonds JS. Determination of arsenic species in environmental and biological samples. Pure Appl Chem 64:575–590 (1992).
6. Cullen WR, Reimer KJ. Arsenic speciation in the environment. Chem Rev 89:713–764 (1989).
7. Maher MA, Batley GE. Organometallics in the nearshore marine environment of Australia. Appl Organomet Chem 4:419–437 (1990).
8. Morita M, Shibata Y. Isolation and identification of an arseno-lipid from a brown alga *Undaria pinnafida* (wakami). Chemosphere 17:1147–1152 (1988).
9. Le X-C, Cullen WR, Reimer KJ. Human urinary arsenic excretion after one-time ingestion of seaweed, crab, and shrimp. Clin Chem 40:617–624 (1994).
10. Pyles RA, Woolson EA. Quantitation and characterization of arsenic compounds in vegetables grown in arsenic acid treated soil. J Agric Food Chem 30:866–870 (1982).
11. Weiler RR. Percentage of inorganic arsenic in food: a preliminary analysis. Report no. 87-48-45000-057. Toronto, Canada:Ontario, Canada Ministry of the Environment, 1987.
12. U.S. EPA. Special report on ingested inorganic arsenic. Skin cancer, nutritional essentiality. EPA/625/3-87/013. Washington:Environmental Protection Agency, 1988.
13. Schoof RA, Yost LJ. Arsenic intake from the Taiwanese diet [abstract]. Fundam Appl Toxicol 30(suppl):49 (1996).
14. Tseng W-P, Chu HM, How SW, Fong JM, Lin CS, Yeh S. Prevalence of skin cancer in an endemic area of chronic arsenism in Taiwan. J Natl Cancer Inst 40:453–463 (1968).
15. Tseng W-P. Effects and dose–response relationships of skin cancer and blackfoot disease with arsenic. Environ Health Perspect 19:109–119 (1977).
16. U.S. EPA. Health assessment document for inorganic arsenic. Final report. Report no. EPA-600/8-83-021F. Washington:Office of Health and Environmental Assessment, 1984.
17. Smith AH, Hopenhayn-Rich C, Bates MN, Goeden HM, Hertz-Picciotto I, Duggan HM, Wood R, Kosnett MJ, Smith MT. Cancer risks from arsenic in drinking water. Environ Health Perspect 97:259–267 (1992).
18. Guthrie HA. Introductory nutrition. In: Water. St. Louis, MO:C.V. Mosby, 1983;213–221.
19. Ershow AG, Brown LM, Cantor KP. Intake of tapwater and total water by pregnant and lactating women. Am J Publ Health 81:328–334 (1991).
20. Kristal-Boneh W, Glusman JG, Cassuto Y. Improved thermoregulation caused by forced water intake in human desert dwellers. Eur J Appl Physiol 57:220–224 (1988).
21. Phillip M, Chaimovitz C, Singer A, Golinsky D. Urine osmolality in nursery school children in a hot climate. Isr J Med Sci. 29:104–106 (1993).
22. Galagan DJ, Vermillion JR, Nevitt GA, Stadt ZM, Dart RE. Climate and fluid intake. Public Health Rep 72:484–490 (1957).
23. Valentine JL, Kang HK, Spivey G. Arsenic levels in human blood, urine and hair in response to exposure via drinking water. Environ Res 20:24–32 (1979).
24. Mushak P, Dessauer K, Walls EL. Flameless atomic absorption (FAA) and gas–liquid chromatographic studies in arsenic bioanalysis. Environ Health Perspect 19:5–10 (1977).
25. Fitchett AW, Daughtrey EH Jr, Mushak P. Quantitative measurements of inorganic and organic arsenic by flameless atomic absorption spectrometry. Anal Chim Acta 79: 93–99 (1975).
26. Daughtrey EH Jr, Fitchett AW, Mushak P. Quantitative measurements of inorganic and methyl arsenicals by gas–liquid chromatography. Anal Chim Acta 79:199–206 (1975).
27. Kang NK, Valentine JL. Acid interference in the determination of arsenic by atomic absorption spectrometry. Anal Chem 49:1829–1832 (1977).
28. Braman RS. Applications of arsine evolution methods to environmental analyses. Environ Health Perspect 19:1–4 (1977).
29. Eaton AD. Determining the practical quantitation level for arsenic. J Am Water Works Assoc 86(2):100–114 (1994).
30. Irgolic KJ. Determination of total arsenic and arsenic compounds in drinking water. In: Arsenic exposure and health (Chappell WR, Abernathy CO, Cothern CR, eds). Environ Geochem Health 16(special issue):51–60 (1994).
31. Irgolic KJ. Arsenic. In: Techniques and instrumentation in analytical chemistry: hazardous metals in the environment, vol.12 (Stoeppler M, ed). Amsterdam:Elsevier Science Publishers, 1992;287–350.
32. Yamauchi H, Takahashi, K, Mashiko M, Yamamura Y. Biological monitoring of arsenic exposure of gallium arsenide- and inorganic arsenic-exposed workers by determination of inorganic arsenic and its metabolites in urine and hair. Am Ind Hyg Assoc J 50:606–612 (1989).
33. Vahter M. Environmental and occupational exposure to inorganic arsenic. Acta Pharmacol Toxicol 59:31–34 (1986).
34. Del Razo LM, Garcia-Vargas G, Vargas G, Vargas H, Albores A, Cebrian E, Montero R, Ostrosky P, Kelch M. Chronic high arsenic exposure alters the profile of urinary arsenic metabolites in humans. Toxicology 15:262 (1995).
35. Hopenhayn-Rich C, Biggs ML, Smith AH, Moore LE, Kalman DA. Arsenic exposure in a chronically exposed population: a multifactorial analysis of metabolite distribution. Presented at the Society for Environmental Geochemistry and Health Second International Conference on Arsenic Exposure and Health Effects, San Diego, CA, 12–14 June 1995.
36. Hopenhayn-Rich C, Biggs ML, Smith AH, Kalman DA, Moore LE. Methylation study of a population environmentally exposed to arsenic in drinking water. Environ Health Perspect 104:620–628 (1996).
37. Warner ML, Moore LE, Smith MT, Kalman DA, Fanning E, Smith AH. Increased micronuclei in exfoliated bladder cells of individuals who chronically ingest arsenic-contaminated water in Nevada. Cancer Epidemiol Biomarkers Prev 3:583–590 (1994).
38. Hughes MF, Menache M, Thompson DJ. Dose-dependent disposition of sodium arsenate in mice following acute oral exposure. Fundam Appl Toxicol 22:80–89 (1994).
39. Thompson DJ. A chemical hypothesis for arsenic methylation in mammals. Chem-Biol Interact 88:89–114 (1993).
40. Vahter M, Marafante E. Effects of low dietary intake of methionine, choline, or proteins on the biotransformation of arsenite in the rabbit. Toxicol Lett 37:41–46 (1987).
41. Mushak P. Mammalian biotransformation processes involving various toxic metalloids and metals. In: Chemical toxicology and clinical chemistry of metals (Brown SS, Savory J, eds). New York:Academic Press, 1983;227–245.